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TITLE: The Effect of Recombinant Factor VIIa and Fibrinogen on Bleeding From Grade V Liver Injuries in Coagulopathic Swine

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13. ABSTRACT (Maximum 200 Words)

This was a 2 part study. The first part of the study was performed to determine if recombinant factor VIIa would reduce bleeding after a grade V liver injury in hypothermic, dilutionally coagulopathic pigs when used as an adjunct to abdominal packing and to determine the optimal dose of the drug. The second part of the study was performed to compare different fluids for resuscitation of this model. Fluids were compared for their effects on volume of resuscitation, rebleeding, metabolic effects and their effects on coagulation. The grade V liver injury model is well described and a clamp is utilized creating a reproducible injury. In part 1 of the study animals underwent a 60% of blood volume isovolemic exchange transfusion with 5% human albumin and the animals' temperature was maintained at 33°C. In part 2, animals were normothermic and non-coagulopathic. The study revealed that rFVIIa reduces blood loss form the grade V injury by approximately 50%. This effect was not changed by increasing the dose form 180 µg/kg to 720 µg/kg. In the second part of the study, we showed that resuscitation with normal saline requires 3 times as much fluid and results in severe acidosis and coagulopathy compared to resuscitation with lactated Ringers solution.

14. SUBJECT TERMS
Recombinant factor VIIa, liver injury, hemorrhage, swine, lactated ringers, normal saline, Hextend

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Table of Contents

INTRODUCTION:

The majority of deaths following combat are secondary to hemorrhage. The goal of this research is to elucidate new methods of hemorrhage control that can be applied to the injured soldier in the field and hospital. This proposal was designed to determine the effectiveness of rFVIIa in reducing hemorrhage after a Grade V liver injury in hypothermic and coagulopathic swine and to determine if increasing the dose of rFVIIa would increase its effectiveness. We had originally intended to use rFVIIa in conjunction with fibrinogen, however our preliminary data revealed that rFVIIa was not effective in reducing blood loss after Grade V injury in swine when used as a sole agent in non-coagulopathic pigs.[1] Therefore, we chose to test its effectiveness when used as an adjunct to abdominal packing in cold, coagulopathic pigs and to determine if there was a dose response. Fluid resuscitation following injury and hemorrhage is also a critical determinant of outcome. In the second part of this study, the same grade V liver injury model was used to study the effects of resuscitation with lactated Ringers solution, normal saline and Hextend. Effects of these solutions on fluid requirement, rebleeding, metabolic changes and coagulation were studied.

BODY:

Materials and Methods

Part 1

Thirty Yorkshire crossbred swine, weighing approximately 30 kg, were utilized. All animals were free of disease and in apparent excellent health. Animals were allowed free access to water and to a commercial laboratory swine food. Food was held the night before the study. All animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care International accredited facility, and all experimental manipulations were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

The swine were anesthetized with an intramuscular (IM) injection of 4.4 mg/kg of Telezol. The animals were also given 0.25 mg/kg of glycopyrrolate IM. They were then intubated with a 7 mm Mallinckrodt endotracheal tube and placed on mechanical ventilation with settings of 10 ml/kg tidal volume, a rate of 12-14 breaths per minute and 100% oxygen. Anesthesia was maintained using 2% isoflurane and an esophageal temperature probe was placed.

Once the animals were fully anesthetized, cutdowns were performed and polyethylene tubing was placed in the left external jugular vein and left common carotid artery. The venous line was used for study drug infusion and fluid resuscitation. The arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure and heart rate were recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer. (Micro-Med®, Louisville, KY).

The animals underwent midline laparotomy, Foley catheter placement and splenectomy. The spleen was weighed and lactated Ringer's (LR) solution at room temperature was infused at 100 ml/min to replace 3 times the spleen weight. Animals

then underwent an isovolemic, exchange transfusion with 5% human albumin at room temperature. An estimated 60% of the animals' blood volume was removed via controlled hemorrhage from the carotid arterial line. The equation Blood volume (ml/kg) = 161.4751(body weight$^{-0.2197}$) was used. The animals' temperature was then standardized to an esophageal temperature of 33°C. This was done by lavaging the abdomen with room temperature lactated Ringer's solution.

Pre weighed laparotomy pads were placed in both gutters and the pelvis to facilitate blood collection. A standardized Grade V liver injury was made with a specially designed liver clamp. For the purposes of this model, a Grade V injury is defined as an injury to a central hepatic vein. This is consistent with the definition of a Grade V injury as indicated by the American Association for the Surgery of Trauma Organ Injury Scaling system.[2]

The current animal model is based upon our experience in previous studies of hemorrhage control utilizing the grade V liver injury model.[3-5] The clamp was positioned in the middle of the liver placing the right hepatic vein, left hepatic vein and portal vein at risk for injury. Blood loss was collected by suction. Thirty seconds after injury, blinded therapy consisting of either 180 μg/kg of rFVIIa, 720 μg/kg or the equivalent amount of buffer solution was infused. Simultaneously, the liver injury was packed with laparotomy sponges and resuscitation with lactated Ringer's solution at 100 ml/min was initiated. The temperature of the resuscitation fluid was varied to maintain a core temperature 33°C. The abdomen was then closed. Animals were resuscitated to their baseline MAP. Pre-treatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the pre weighed laparotomy pads before and after bleeding.

The study was continued for 2 hours from the time of injury. During this time period, lactated Ringer's (LR) solution was given as needed to maintain the baseline MAP. The core temperature was maintained at 33°C by varying the fluid temperature and by using a Bair Hugger warming system (Eden Prairie, MN). Time of death was recorded for those animals that did not survive the 2-hour study period.

After 2 hours, the animals were euthanized and the abdomen was opened. Free blood in the abdomen was suctioned and the packing laparotomy sponges were weighed. Post treatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the laparotomy sponges from before placement to the end of the study. An autopsy of the liver was performed to insure that injuries were comparable between groups.

Laboratory studies were drawn at baseline, prior to injury, 5 minutes after injury, 1 hour after injury and at the end of the study. Laboratory studies included arterial blood gas, complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, thrombin anti-thrombin complexes and d-dimers. Coagulation studies were performed at the temperature at which they were drawn.

Part 2

Yorkshire crossbred swine weighing a mean of 36 kg were utilized. Anesthesia protocols, instrumentation and operative procedures were identical to Part 1 except animals did not undergo dilution and hypothermia and injuries were allowed to bleed

freely without packing. In the initial stage of part 2 animals were assigned to receive resuscitation with either LR or normal saline (NS). In the second stage of part 2 animals were assigned to receive no resuscitation, LR or Hextend which is 6% hetastarch in a balanced salt solution. Resuscitation was initiated 30 minutes after injury at 165 cc/min with a goal of reaching and maintaining the baseline blood pressure for a total of 120 minutes after injury. Laboratory studies were performed at baseline, 30 minutes after injury, 60 minutes after injury, 90 minutes after injury and at the conclusion of the study except for thrombelastograms (TEGs) which were performed at baseline and just prior to euthanasia. Following completion of the study, lung tissue was harvested for cytokine analysis and myeloperoxidase staining.

Statistical Analysis

The paired t-test was used to compare means of matched continuous variables. Dichotomous data were analyzed with the chi-square test. If the value of a categorical variable in any cell was less than five, Fisher's exact test was utilized. Statistical analysis was performed using commercially available software from Stata Corporation, College Station, Texas. Statistical significance was defined as a p-value < 0.05.

Results

Part 1

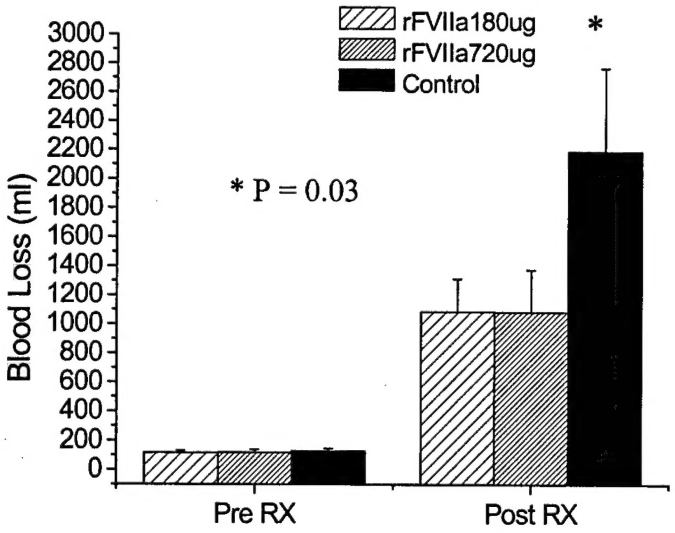
Ten animals randomized to each of the 3 groups. Mean weight, temperature, number of vessels injured and fluid resuscitation are shown in Table 1. Weight and temperature were similar between groups as was the severity of injury. Animals in the control group received approximately twice as much resuscitation fluid as animals in the 2 treatment groups. However, due to large standard deviations, these differences were not statistically significant.

Table 1.

	RFVIIa 180 ug/kg	RFVIIa 720 ug/kg	Control	P
Mean Weight (kg)	31.4	31.7	31.6	NS
Mean Injury Temp (°C)	33.1	33.3	33.2	NS
Mean Vessels Injured	2	2.1	2.1	NS
Mean Fluid Resuscitation (ml)	2717	2734	4514	0.2

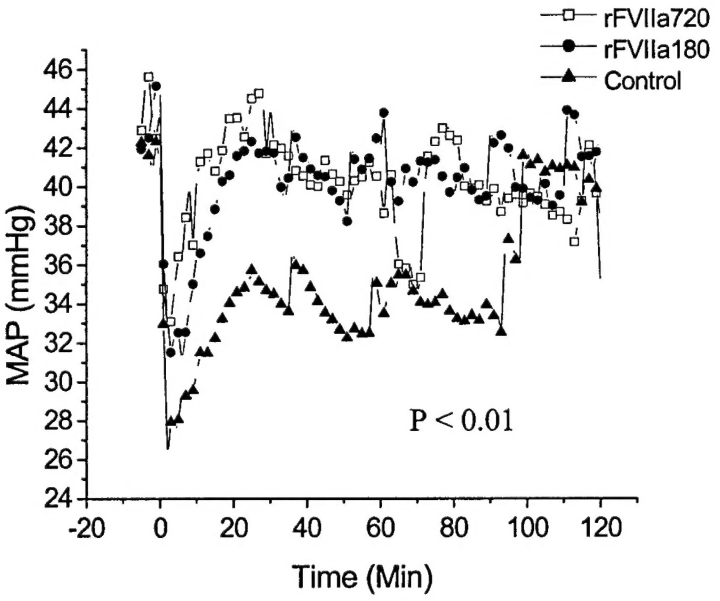
Blood loss after treatment in the control group was approximately twice that of either treatment group. This difference was significant at the $p = 0.03$ level. There was no difference in blood loss between the 2 treatment groups. This data is shown in Figure 1.

Figure 1. Blood loss pre and post treatment.



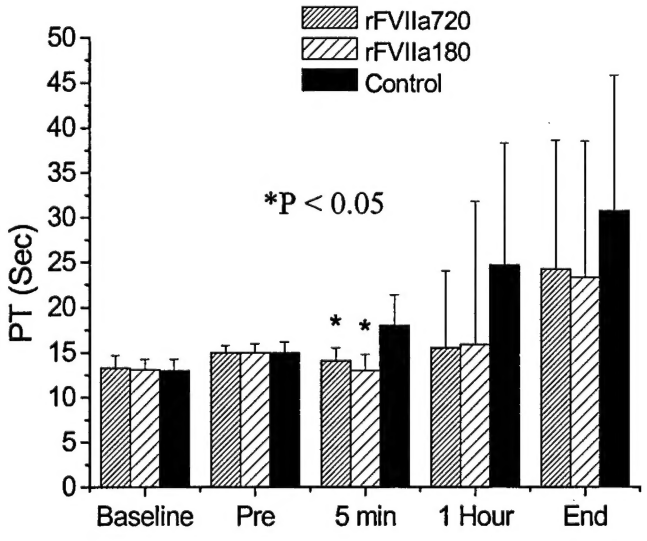
The mean arterial pressures of the 3 groups are shown in Figure 2. The control group had a low mean nadir MAP and the blood pressure of the control group was significantly lower than the treatment groups throughout the study. There was no difference between the 2 treatment groups.

Figure 2. Mean arterial pressure over the course of the study.



Mean prothrombin times measured over the course of the study are shown in Figure 3. Following treatment, there was a significant reduction in the prothrombin times in the 2 treatment groups as compared to the control group. This difference was no longer significant 1 hour after injury or at the end of the study.

Figure 3. Prothrombin time



Partial thromboplastin times were not significantly different over the course of the study between groups.

Figures 4 and 5 show thrombin antithrombin complexes (TATs) and d-dimers measured serially between groups. Both groups that received rFVIIa exhibited significantly elevated TATs compared to the control group. These differences were present 5 minutes after treatment and throughout the remainder of the study. Similarly, d-dimers were significantly elevated in the treatment groups relative to the control group. These differences were present at 1 hour and at the end of the study.

Figure 4. Thrombin antithrombin complexes

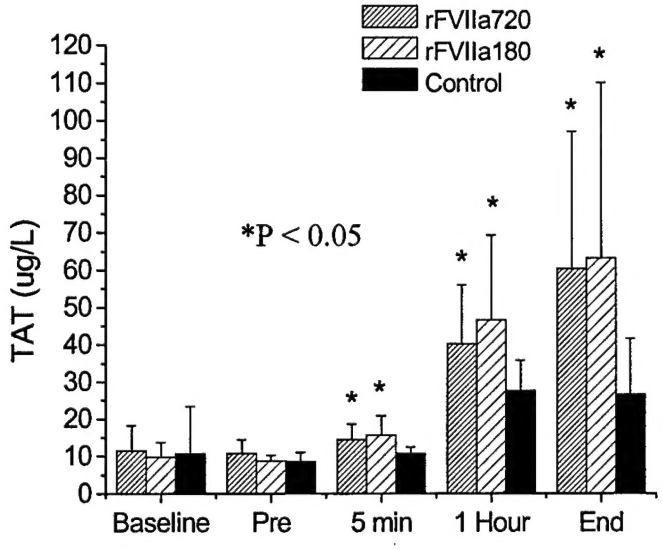
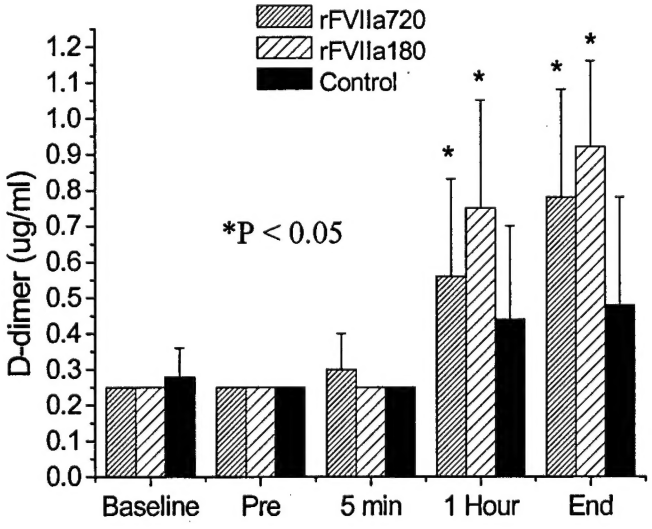


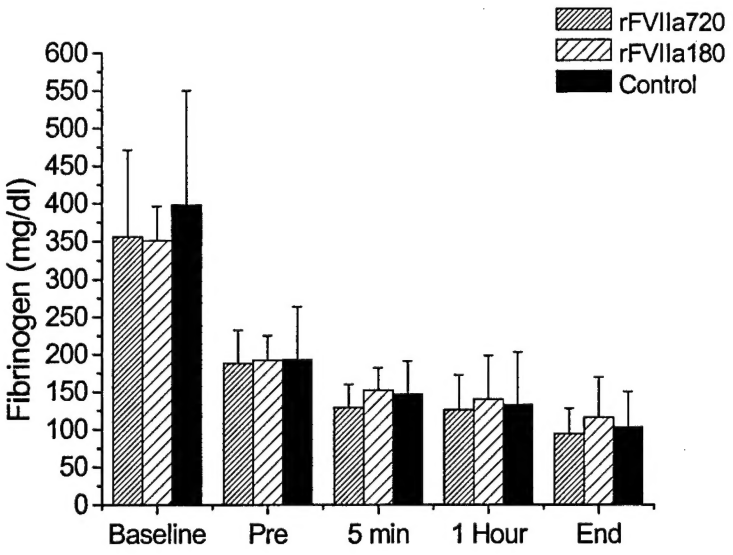
Figure 5. D-dimers



Despite the observed elevations in TATs and d-dimers, there was no decrease in either fibrinogen levels (Figure 6) or platelets in the treatment groups as compared to the control group. This suggests that rFVIIa did not induce the syndrome of disseminated intravascular coagulation or activate systemic coagulation. The elevations of TATs and

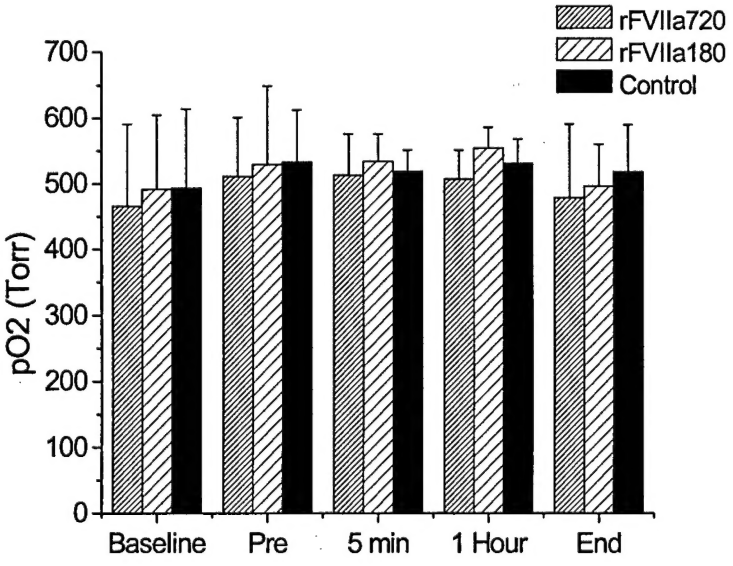
d-dimers are hypothesized to be secondary to increased activation of localized clotting activity at the site of injury. It should be noted that a human latex agglutination test was used to measure d-dimers. The validity of using human antibodies to detect pig d-dimers is unknown.

Figure 6. Fibrinogen levels.



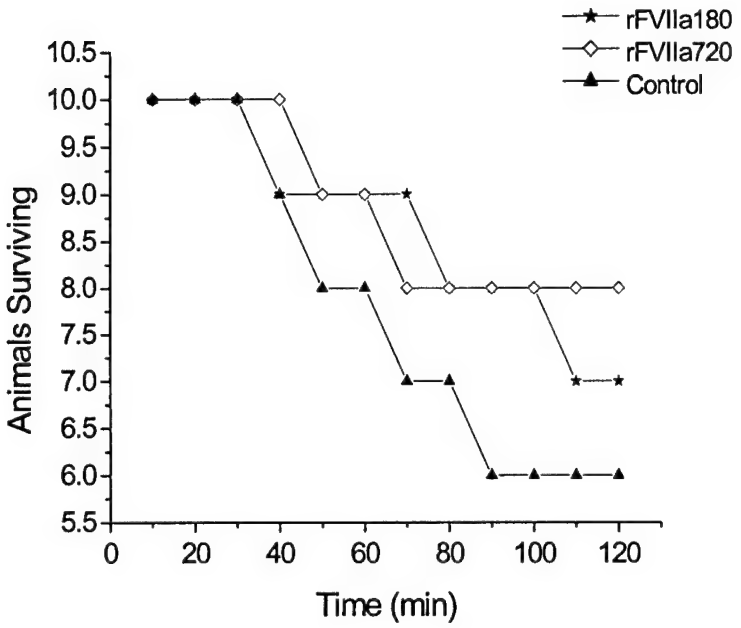
The prior study from our laboratory showed a trend toward increased pulmonary capillary microthrombosis in animals receiving rFVIIa. Histology studies from this study have not yet been concluded however there was no evidence of decreased oxygenation in animals receiving rFVIIa. (Figure 7)

Figure 7. Oxygenation over the course of the study.



Survival during the 2 hours study was 6 out of 10 in the control group, 7 out of 10 in the 180 group and 8 out of 10 in the 720 group. There was no statistical difference in overall survival or mean survival time between the 3 groups. (Figure 8)

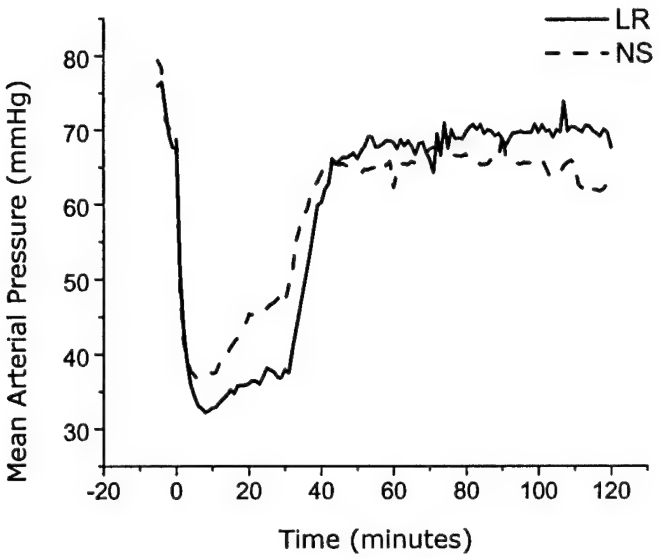
Figure 8. Survival



Part 2
LR vs NS

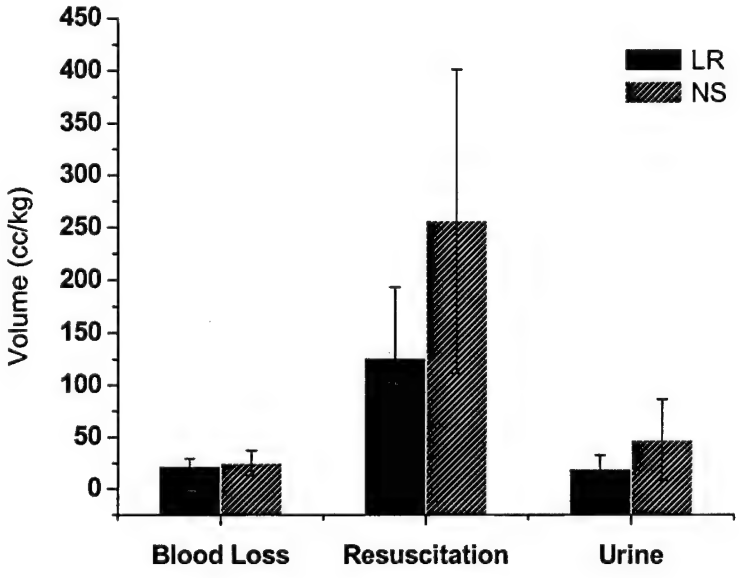
10 animals randomized to each group. Initial blood loss was 22 cc/kg in the LR group and 25 cc/kg in the NS group. ($p = 0.54$) Blood pressures over the course of the study are shown in Figure 9 and are not statistically different.

Figure 9. Mean arterial pressure after injury and resuscitation.



Animals resuscitated with NS required approximately twice as much fluid as animals resuscitated with LR ($p = 0.02$) and they had greater than twice as much urine output. ($p = 0.04$) These data are summarized in Figure 10. There was no difference in rebleeding blood volumes.

Figure 10. Comparison of blood loss, resuscitation requirements and urine output.



Animals resuscitated with NS had a significant hyperchloremic acidosis and evidence of coagulopathy as indicated by decreased fibrinogen levels. Interestingly animals resuscitated with lactated Ringers had elevated lactate levels that were not associated with an acidosis. (Table 2) This most likely represents measured sodium lactate from the solution itself.

Table 2. Laboratory study between groups.

	Normal Saline	Lactated Ringers	P Value
Sodium	149 +/- 1.0	138 +/- 0.5	< 0.01
Chloride	119 +/- 1.9	105 +/- 2.9	< 0.01
pH	7.28 +/- 0.12	7.45 +/- 0.06	< 0.01
Base Excess	-4.6 +/- 2.6	7.2 +/- 1.3	< 0.01
Lactate	1.7 +/- 0.6	4.7 +/- 0.7	< 0.01
Fibrinogen	99 +/- 7	123 +/- 7	0.01

Lung tissue levels of IL-6, G-CSF and TNF-α mRNA as well as the results of MPO staining are shown in Table 3. There was no difference in cytokine expression between the 2 groups. The number of neutrophils per high power field were elevated in the LR and NS groups as compared to controls and shams but were not different when compared to each other.

Table 3. Inflammatory response

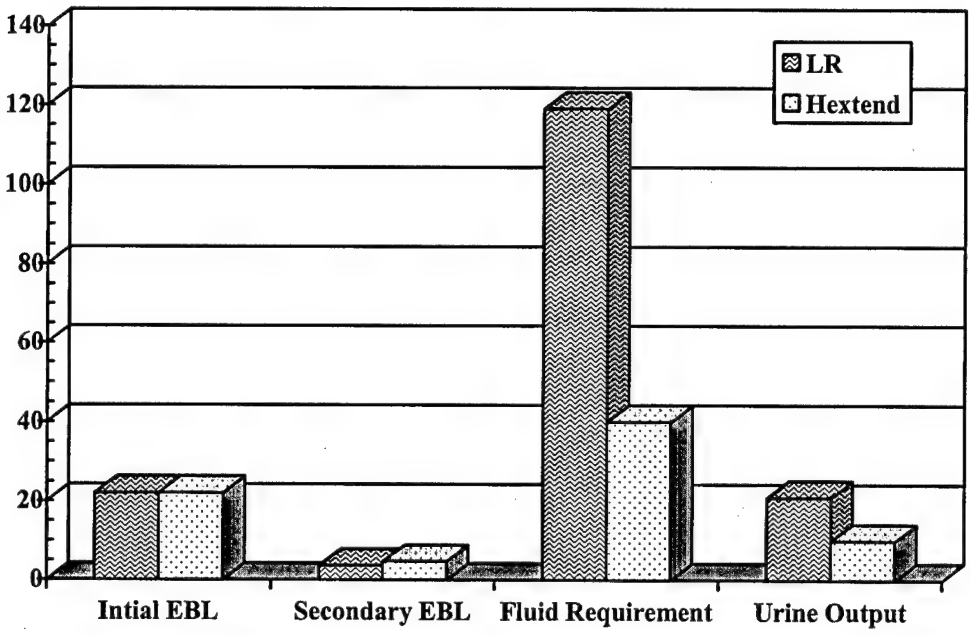
Cytokine mRNA Expression			
	IL-6	G-CSF	TNF-alpha
LR	0.06 $\pm$ 0.06	4.8 $\pm$ 4.3	8.4 $\pm$ 5.8
NS	0.06 $\pm$0.04	5 $\pm$10.8	4.8 $\pm$ 2.9
p-value	0.99	0.96	0.1

MPO Staining	
	PMNs/hpf
Control	6.9 $\pm$ 1.3
Sham	11.8 $\pm$ 1.6
LR	17.2 $\pm$ 6.9
NS	16.6 $\pm$ 4.9

LR vs. Hextend and non-Resuscitated Animals

10 animals randomized to each of the resuscitation groups and there were 6 animals in the non-resuscitated group. Initial blood loss was 22 cc/kg in each group. Animals resuscitated with LR required approximately 3 times as fluid to reach and maintain their baseline blood pressure. Urine output was also significantly greater in the LR group. There was no difference in rebleeding volume. These data are summarized in Figure 11.

Figure 11. Blood loss, fluid requirement and urine output between groups



Pertinent laboratory values along with their standard deviations upon study completion are presented in Table 4. As seen here, there were significant differences between the hemoglobin and hematocrit values in the two treatment groups versus the control group. However, there were no differences in these values between the two treatment groups.

The standard tests of coagulation (platelets, PTT, PT, and fibrinogen) were similar between the control group and the LR group. In contrast these values were significantly different between the Hextend animals and the other two groups. These values reveal that the Hextend group trended toward a hypocoagulable state.

Table 4. Laboratory values at the end of the study

	CG	LR	HEX	*p* Values		
				LRv.CG	HEXv.CG	LRv.HEX
Hemoglobin (g/dL)	8.4+/-0.5	5.1+/-0.9	4.5+/-1.2	<0.01	<0.01	NS
Hematocrit (%)	25.2+/-1.6	14.8+/-2.8	13.6+/-3.1	<0.01	<0.01	NS
Platelets ($10^3/\mu L$)	392+/-55	322+/-92	165+/-98	NS	<0.01	<0.01
PTT (sec)	16.7+/-3.5	16+/-2.3	22.1+/-6.6	NS	0.05	0.02

PT (sec)	12.4+/-0.4	14.2+/-0.7	15.7+/-1.1	<0.01	<0.01	<0.01
Fibrinogen (mg/dL)	210+/-52	161+/-42	112+/-33	NS	<0.01	<0.01

The TEG values upon study completion are presented in Table 5. For both the control (which received no fluid resuscitation) and LR animals, these values all represent significant hypercoagulability. The R value for the Hextend animals reveals hypercoagulability, yet significantly less so than the other two groups. The α angle and MA were within normal limits.

Table 5. TEG values at the end of the study.

	Normal Values	CG	LR	HEX	*p* Values		
					LRv.CG	HEXv.CG	LRv.HEX
R Value (min)	3.7-8.3	1.7+/-0.8	1.7+/-0.6	2.8+/-1.1	NS	0.05	0.02
α Angle (deg)	46.8-73.6	81.1+/-1.1	81.4+/-1.3	65.4+/-11.1	NS	<0.01	<0.01
MA (mm)	54.5-72.5	76.2+/-3.7	75.0+/-3.8	56.4+/-15.0	NS	<0.01	<0.01

CG, control group; LR, lactated Ringer's; HEX, Hextend; MA, maximum amplitude

KEY RESEARCH ACCOMPLISHMENTS:

- Recombinant Factor VIIa reduces blood loss in hypothermic, dilutionally coagulopathic pigs with Grade V liver injuries when used as an adjunct to liver packing.
- Quadrupling the dose of rFVIIa does not increase its efficacy or alter its physiologic effects in any of the measured parameters.
- The infusion of rFVIIa results in the elevation of thrombin antithrombin complexes and d-dimers in severely injured animals. However, there is no evidence of associated disseminated intravascular coagulation.
- Resuscitation with NS results in a significantly greater volume requirement and urine output compared to resuscitation with LR.
- Resuscitation with NS results in hyperchloremic acidosis, while resuscitation with LR results in elevated lactate levels not associated with acidosis.
- Resuscitation with LR and NS produce similar inflammatory profiles with respect to cytokine measurements and myeloperoxidase staining.

- Resuscitation with Hextend results in 1/3 the volume requirement compared to resuscitation with LR.
- Grade V liver injury and hemorrhage without resuscitation results in a hypercoagulable state as measured by TEG. This hypercoagulable state is unaffected by resuscitation with LR but it is partially ameliorated by resuscitation with Hextend without resulting in increased rebleeding volumes.

REPORTABLE OUTCOMES:

Part 1

This research was presented at the 2001 Advanced Technology Applications to Combat Casualty Care conference in Ft Walton Beach, Florida. This work has also been presented at the Eastern Association for the Surgery of Trauma meeting which was held January 17 – 19, 2002 in Orlando, Florida. The work was published in the August 2002 version of the Journal of Trauma. A reprint of the publication is attached to this report. (Appendix 1)

Part 2

The comparison between LR and NS was presented at the 26th Annual Conference on Shock in Phoenix, Arizona and published in abstract form in Shock.[6] A copy of the abstract is attached to this report. (Appendix 2) The comparisons in inflammatory response between LR and NS have been accepted for presentation at the Society of University Surgeons 2004 meeting in St. Louis, Missouri. The abstract is attached as Appendix 3. The manuscript will be submitted to Surgery on 1/16/04. The comparison between LR and Hextend has been accepted for presentation at the Eastern Association for the Surgery of Trauma 2004 meeting in Orlando, Florida. The abstract will be published in the January issue of Journal of Trauma and the manuscript has been submitted for publication. The abstract is attached as Appendix 4.

CONCLUSIONS:

This work confirms that rFVIIa is effective as adjunctive therapy in a severe liver injury model in hypothermic, dilutionally coagulopathic pigs. This work is complementary to our prior study which showed no effect of rFVIIa in non-coagulopathic pigs with the same liver injury when the drug was used as sole therapy.[1] Combining the data from the 2 studies suggests that rFVIIa should be used as an adjunct to standard surgical therapy to control the bleeding of coagulopathy and hypothermia.

Increasing the dose of rFVIIa by 4 times did not increase its efficacy. This is most likely explained by the mechanism of action of the drug. Factor VIIa functions by binding exposed and activated tissue factor. It is likely that the dose of 180 μg/kg was adequate to saturate the available exposed and activated tissue factor. Therefore, increasing the dose of the drug had no additional effect.

Lactated Ringers solution is superior to normal saline for the resuscitation of uncontrolled hemorrhagic shock. Resuscitation with LR results in significantly decreased

volume requirements and less metabolic derangements to include acidosis and coagulopathy. Contrary to prior reports resuscitation with LR does not increase dysfunctional inflammation.

Injury and hemorrhage without resuscitation produce a hypercoagulable state. This hypercoagulable state is not affected by resuscitation with LR but it is attenuated by resuscitation with Hextend without increasing blood loss. Outcomes after trauma may be improved by using resuscitation fluids that modulate patient coagulability status and alter the inflammatory response to trauma.

Future research evaluating the ideal resuscitation regimen following uncontrolled hemorrhagic shock is indicated. The ideal fluid as well as the ideal fluid resuscitation endpoint should be evaluated. Other fluids which may prove beneficial include hypertonic saline and hemoglobin substitutes.

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APPENDIX 1

The Effect of Recombinant Factor VIIa on Coagulopathic Pigs with Grade V Liver Injuries

Martin A. Schreiber, MD, FACS, John B. Holcomb, MD, FACS, Ulla Hedner, MD, Susan I. Brundage, MD, FACS, Joseph M. Macaitis, BS, and Keith Hoots, MD

Background: **Recombinant factor VIIa (rFVIIa) has been used to decrease bleeding in a number of settings including hemophilia, liver transplantation, intractable bleeding, and cirrhosis. Experience in the trauma setting is limited. This study was performed to determine whether rFVIIa would reduce bleeding after a grade V liver injury in hypothermic, dilutionally coagulopathic pigs when used as an adjunct to abdominal packing and to determine whether increasing the dose of the drug increased its hemostatic efficacy.**

Methods: **Thirty animals were randomized to receive 180 μg/kg of rFVIIa, 720 μg/kg of rFVIIa, or vehicle buffer control. After laparotomy and splenectomy, animals underwent a 60% blood volume isovolemic exchange transfusion with 5% human albumin. The animals' temperature was maintained at 33°C and a standardized grade V liver injury was made with a liver clamp. Thirty seconds after injury, the abdomen was packed with laparotomy sponges, resuscitation was initiated, and blinded therapy was given. Animals were resuscitated to their baseline mean arterial pressure and the study was continued for 2 hours. Serial coagulation parameters were measured at the temperature they were drawn. After the study period, surviving animals were killed, posttreatment blood loss was measured, and an autopsy was performed.**

Results: **Ten animals were randomized to each group. After administration of study drug, factor VII clotting activity (FVII:C) was higher in the 720-μg/kg group than in the 180-μg/kg group ($p < 0.01$). FVII:C was higher in both treatment groups than in the control group ($p < 0.01$). The mean prothrombin time was shorter in the treatment groups than in the control group ($p < 0.05$). Mean arterial pressure was lower in the control group than in the treatment groups throughout the study ($p < 0.01$). Mean blood loss was less in the treatment groups than in the control group ($p = 0.03$). Mortality was not different between groups. There were no differences between the groups that received rFVIIa in any measured parameters except for FVII:C. Liver injuries were similar between groups and there was no evidence of microthrombosis on lung histology.**

Conclusion: **rFVIIa reduces blood loss in hypothermic, dilutionally coagulopathic pigs with grade V injuries when used as an adjunct to packing. Increasing the dose does not enhance the hemostatic effect.**

Key Words: **Recombinant factor VIIa, Liver injury, Swine, Hemorrhage, Hypothermia, Coagulopathy.**

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Recombinant factor VIIa (rFVIIa) has been described as a universal hemostatic agent.[1] It was originally designed to treat hemophiliacs with inhibitors to factor VIII and factor IX,[2,3] and it is approved by the Food and Drug Administration for that purpose. Successful use of the drug in patients with platelet disorders, cirrhosis, intractable bleeding from various causes, liver transplantation, and cardiac surgery has also been published.[4–11]

The published uses of rFVIIa in the clinical trauma setting have been limited, and consist of a case report and a small, uncontrolled case series. The case report involved an Israeli soldier who sustained a high-velocity rifle injury to his inferior vena cava. The patient appeared to be moribund despite maximum standard efforts, but intravenous infusion of rFVIIa resulted in arrest of blood loss and the patient survived.[12] The case series consisted of seven massively injured patients whose median transfusion requirement was 40 units. rFVIIa was used as an adjunct to standard surgical techniques. The administration of rFVIIa resulted in correction of coagulopathy and cessation of diffuse bleeding in all of the patients. Three of the seven patients died from reasons other than bleeding or thromboembolism.[13]

The use of rFVIIa in pigs with grade V liver injuries has also been described. When used as sole therapy in warm noncoagulopathic animals, a dose of 150 μg/kg has been shown to be ineffective in decreasing blood loss.[14] However, in a small series of 10 hypothermic and diluted animals with the same injury, 180 μg/kg of rFVIIa was shown to significantly reduce blood loss as compared with normal saline

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control when used as an adjunct to abdominal packing.[15] The effect of dose on hemostatic efficacy in the pig liver injury model has not been studied. This study was performed to confirm the efficacy of rFVIIa in reducing blood loss from grade V liver injuries in cold coagulopathic pigs when used as an adjunct to packing and to determine whether quadrupling the dose of the drug would increase its efficacy.

MATERIALS AND METHODS

Thirty Yorkshire crossbred swine of both sexes, weighing approximately 30 kg, were used. All animals were free of disease and in apparent excellent health. Animals were allowed free access to water and to a commercial laboratory swine food. Food was withheld the night before the study. All animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all experimental manipulations were performed in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*. The protocol was approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine.

The swine were anesthetized with an intramuscular injection of 4.4 mg/kg of Telezol. The animals were also given 0.25 mg/kg of glycopyrrolate intramuscularly. They were then intubated with a 7-mm Mallinckrodt endotracheal tube and placed on mechanical ventilation with settings of 10 mL/kg tidal volume, a respiratory rate of 12 to 14 breaths/min, and 100% oxygen. Respiratory rate and tidal volume were adjusted to maintain the Pco_2 close to 40 mm Hg. Anesthesia was maintained using 2% isoflurane and an esophageal temperature probe was placed.

Once the animals were fully anesthetized, cutdowns were performed and polyethylene tubing was placed in the left external jugular vein and left common carotid artery. The venous line was used for study drug infusion and fluid resuscitation. The arterial line was used for continuous blood pressure monitoring and blood sampling. Mean arterial pressure, systolic pressure, diastolic pressure, and heart rate were recorded and averaged every 10 seconds using a digital data collection system with a blood pressure analyzer (Micro-Med, Inc., Louisville, KY).

The animals underwent midline laparotomy, Foley catheter placement, and splenectomy. The spleen was weighed and lactated Ringer's (LR) solution at room temperature was infused at 100 mL/min to replace three times the spleen weight. After splenectomy and spleen replacement, there was a 15-minute stabilization period. Animals then underwent an isovolemic exchange transfusion with 5% human albumin at room temperature. An estimated 60% of the animal's blood volume was removed via controlled hemorrhage from the carotid arterial line. The following equation was used: blood volume (mL/kg) = $161.4751(\text{body weight}^{-0.2197})$.[16] The animal's temperature was then standardized to an esophageal temperature of 33°C. This was done by lavaging the abdomen with room-temperature LR solution. This model for hypothermia and coagulopathy is a modification of the model that was developed by Holcomb et al. to test the efficacy of a dry fibrin sealant dressing, and it is designed to reflect the scenario of massive blood loss and resuscitation seen in severely injured patients.[17]

Preweighed laparotomy pads were placed in both gutters and the pelvis to facilitate blood collection. A standardized grade V liver injury was made with a specially designed liver clamp. For the purposes of this model, a grade V injury is defined as an injury to a central hepatic vein. This is consistent with the definition of a grade V injury as indicated by the American Association for the Surgery of Trauma Organ Injury Scaling system.[18]

The clamp was positioned in the middle of the liver, placing the right hepatic vein, left hepatic vein, and portal vein at risk for injury. Blood loss was collected by suction. Thirty seconds after injury, blinded therapy consisting of either 180 μg/kg of rFVIIa, 720 μg/kg of rFVIIa, or the equivalent amount of buffer solution was infused. Simultaneously, the liver injury was packed with laparotomy sponges, and resuscitation with LR solution at 100 mL/min was initiated. The temperature of the resuscitation fluid was varied to maintain a core temperature 33°C. The abdomen was then closed. Animals were resuscitated to their baseline mean arterial pressure (MAP). Pretreatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the preweighed laparotomy pads before and after bleeding.

The study was continued for 2 hours from the time of injury. During this time period, LR solution was given as needed to maintain the baseline MAP. The core temperature was maintained at 33°C by varying the fluid temperature and by using a Bair Hugger warming system (Augustine Medical, Inc., Eden Prairie, MN). Time of death was recorded for those animals that did not survive the 2-hour study period.

After 2 hours, the animals were killed and the abdomen was opened. Free blood in the abdomen was suctioned and the laparotomy sponges were weighed. Posttreatment blood loss was calculated as the sum of the volume of blood suctioned and the difference in weight of the laparotomy sponges from before placement to the end of the study. A necropsy of the liver was performed to ensure that injuries were comparable between groups and the left lung was removed for histology.

Laboratory studies were drawn at baseline (after splenectomy and spleen replacement), just before injury, 5 minutes after injury, 1 hour after injury, and at the end of the study. Laboratory studies included arterial blood gas, complete blood count, factor VII clotting activity (FVII:C), prothrombin time (PT), partial thromboplastin time, fibrinogen, and thrombin-antithrombin complexes (TATs). Coagulation studies were performed at the temperature at which they were drawn. D-dimers were not used in this study because a reliable test for pigs has not yet been developed and verified.

Table 1 Comparison of Mean Weight, Mean Injury Temperature, Mean Number of Vessels Injured, and Mean Fluid Resuscitation between Groups

	180 μg/kg	720 μg/kg	Control	*p* Value
Mean weight (kg)	31.4 ± 2.8	31.7 ± 3.3	31.6 ± 3.5	NS
Mean injury temperature (°C)	33.1 ± 0.4	33.3 ± 0.2	33.2 ± 0.2	NS
Mean vessels injured	2 ± 0.8	2.1 ± 0.6	2.1 ± 0.7	NS
Mean fluid resuscitation (mL)	2,717 ± 3,324	2,734 ± 2,139	4,514 ± 3,566	0.2

Lung histology was examined by an independent blinded pathologist. Hematoxylin and eosin staining as well as immunostaining for fibrin were performed. Immunostains were prepared using a two-layered method. After antigen retrieval using 1% protease, rabbit anti-human fibrinogen antibody (DAKO A0080 was applied as primary antibody and goat anti-rabbit antibody labeled with horseradish peroxidase (Jackson 111-035-003) as secondary antibody. Vector red served as chromogene and a positive control section was included. As a negative control, an adjacent section was stained as above without the addition of the primary antibody.

Statistical Analysis

The Student's *t* test was used to compare the means of continuous variables. Dichotomous data were analyzed with the χ^2 test. If the value of a categorical variable in any cell was less than 5, Fisher's exact test was used. Statistical analysis was performed using commercially available software from Stata Corporation (College Station, TX). Statistical significance was defined as a value of $p < 0.05$.

RESULTS

Ten animals were randomized to each of the three groups. Mean weight, temperature, number of vessels injured, and fluid resuscitation are shown with their standard deviations in Table 1. Weight and temperature were similar between groups, as was the severity of injury. Animals in the control group received approximately twice as much resuscitation fluid as animals in the two treatment groups. However, because of large standard deviations, these differences were not statistically significant.

Pretreatment blood loss was nearly identical among the three groups. Mean blood loss after treatment was 2,187 mL in the control group versus 1,085 mL in the 180-μg/kg group and 1,086 mL in the 720-μg/kg group. This difference was significant at the $p = 0.03$ level when the two treatment groups were combined and compared with the control group. There was no difference in blood loss between the two treatment groups (Fig. 1).

The mean arterial pressures of the three groups are shown in Figure 2. The 60% isovolemic hemodilution resulted in marked hypotension with a mean preinjury MAP for all animals of 41.7 ± 3.7 mm Hg. After injury, the nadir MAP occurred at a mean of 4.5 ± 3.1 minutes and the average MAP dropped by 37% ± 16%. These values were not different between groups. Throughout the remainder of the study, the mean MAP of the control group was significantly lower than the treatment groups ($p < 0.05$). There was no difference between the two treatment groups.

After treatment, FVII:C increased by 340-fold over baseline in the 720-μg/kg group and by 103-fold in the 180-μg/kg group ($p < 0.01$). FVII:C remained significantly greater in the 720-μg/kg group than in the 180-μg/kg group throughout the remainder of the study and significantly higher in the 180-μg/kg group than in the control group (Fig. 3).

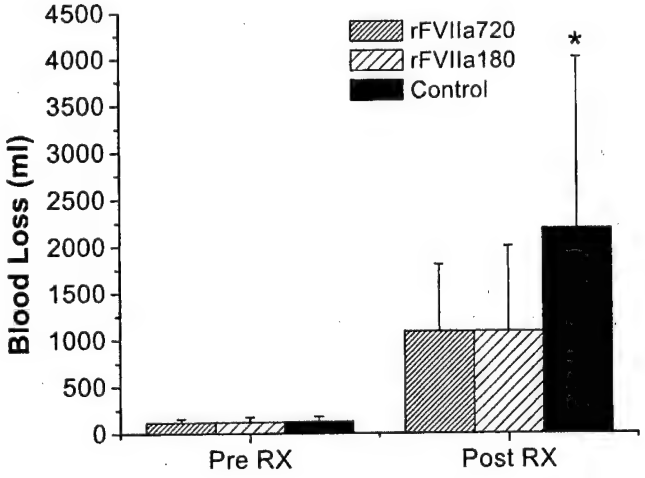

Fig. 1. *Blood loss before and after treatment. *p = 0.03 for the combined treatment groups versus the control group.*

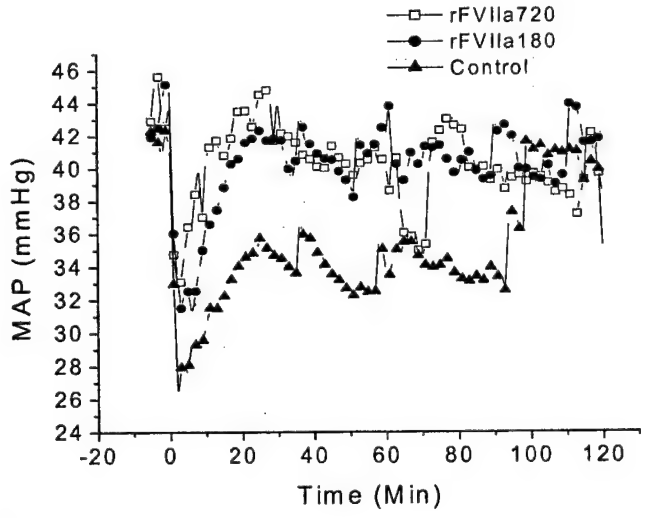

Fig. 2. *Serial mean arterial pressures over the course of the study.*

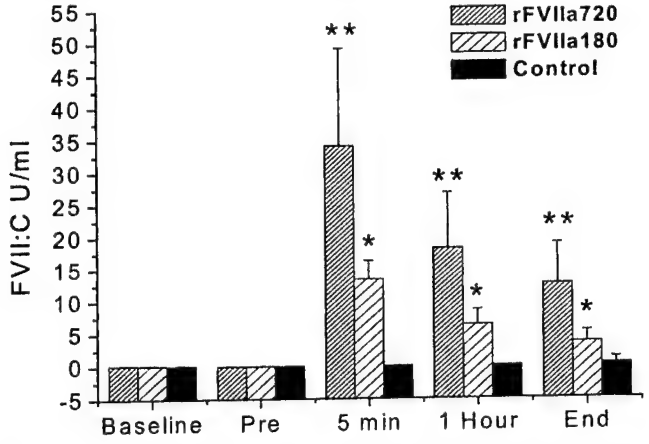

Fig. 3. *Mean factor VII clotting activity (FVII:C). *p < 0.01 for 180 μg/kg versus control; **p < 0.01 for 720 μg/kg versus 180 μg/kg and control.*

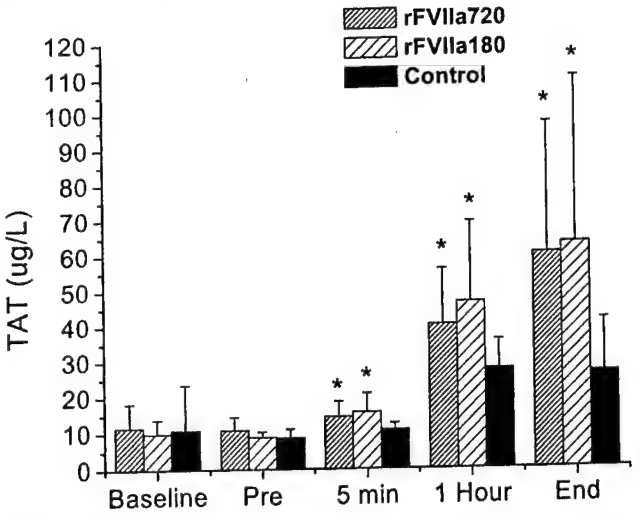

Fig. 5. *Thrombin-antithrombin complexes (TATs). *p < 0.05 for 720 μg/kg versus control and for 180 μg/kg versus control.*

Mean PTs measured over the course of the study are shown in Figure 4. Prothrombin times were measured at the temperature at which they were drawn. After treatment, there was a significant reduction in the PTs in the two treatment groups as compared with the control group. This difference was no longer statistically significant 1 hour after injury or at the end of the study. The PT in the control group at the end of the study was significantly greater than the preinjury PT, probably representing ongoing hemodilution from increased blood loss resulting in decreased MAP, thus necessitating increased resuscitation. Prothrombin values at the end of the study in the treatment groups were not significantly different compared with preinjury values. Partial thromboplastin times were not significantly different over the course of the study between groups.

TATs are compared serially between groups in Figure 5. TATs increased significantly during the observation period in all groups. The groups that received rFVIIa exhibited significantly higher TATs compared with the control group at all time points after treatment, including the 5-minute postinfusion sampling time. TATs were not different between the two treatment groups.

Fibrinogen levels (Fig. 6) and platelet counts (Fig. 7) decreased significantly over the course of the study in all three groups, most likely as a result of dilution. There was no difference in these parameters between groups at any time point. Because of concerns of rFVIIa causing abnormal pulmonary microthrombosis, oxygen tension was measured throughout the study. There was no difference in oxygenation between groups or within groups over the course of the study. Similarly, lung histology revealed no evidence of premorbid microthrombosis or abnormal clotting of the pulmonary vasculature.

Survival during the 2-hour study was 6 of 10 in the control group, 7 of 10 in the 180-μg/kg group, and 8 of 10 in

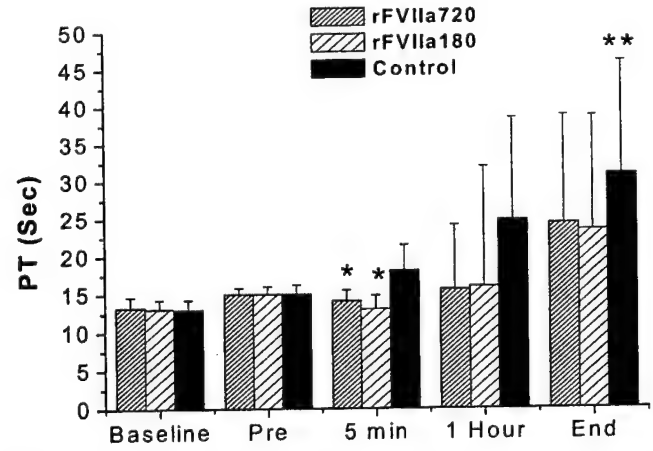

Fig. 4. *Mean prothrombin times. *p < 0.05 for 720 μg/kg versus control and for 180 μg/kg versus control; **p < 0.01 for control at end versus preinjury control.*

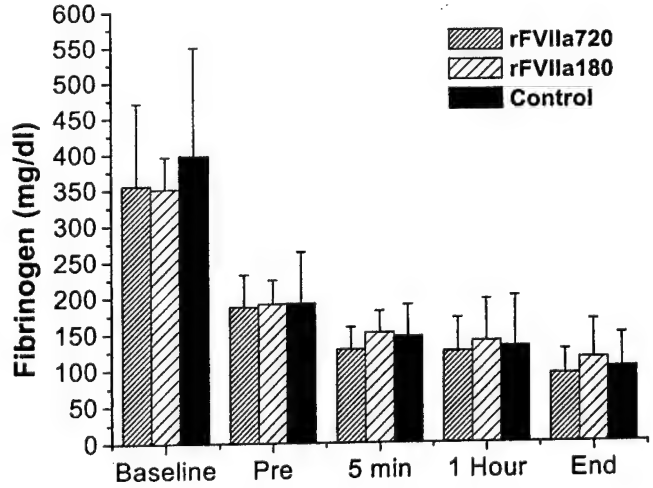

Fig. 6. *Mean fibrinogen levels compared between groups.*

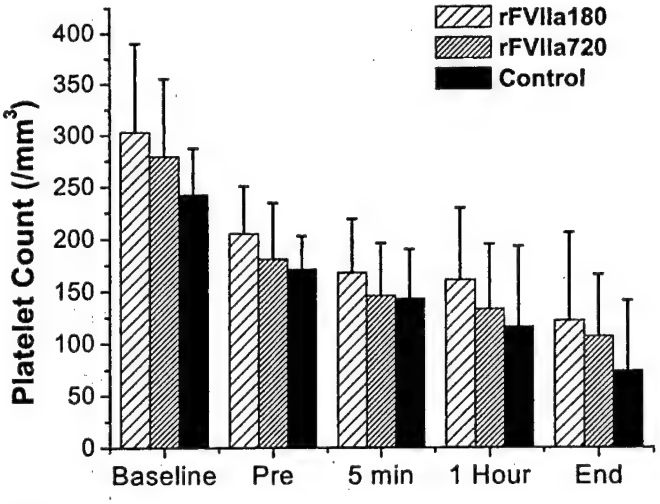

Fig. 7. *Mean platelet counts compared serially over the course of the study.*

the 720-μg/kg group. There was no difference in overall survival or mean survival time between the three groups (Fig. 8).

DISCUSSION

This study confirms the findings of Martinowitz et al. that rFVIIa reduces blood loss in animals with grade V liver injuries when used as an adjunct to liver packing.[15] This study differed from the study by Martinowitz et al. because 5% human albumin was used for the 60% isovolemic hemodilution as opposed to hetastarch. Five percent human albumin was used to avoid the coagulopathy seen with the high-molecular-weight hetastarch that is commonly used in the United States.[19–21]

The hemostatic effect of rFVIIa was not enhanced by quadrupling the dose of the drug despite evidence of the increased dose on the basis of elevated FVII:C. Mean arterial

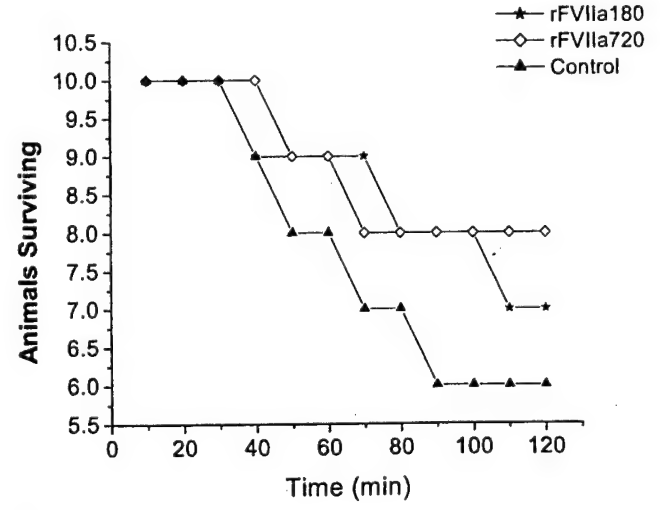

Fig. 8. *Survival curves.*

pressure and other coagulation parameters were also not different between the two treatment groups. The absence of an added effect in the higher dose group may be related to the mechanism of action of the drug. Injury results in exposure and deencryption of tissue factor at the site of injury, which then combines with FVIIa. The FVIIa–tissue factor complex then activates FIX and FX, resulting ultimately in thrombin production and cleavage of fibrinogen to fibrin. Thrombin formation also results in platelet membrane changes, causing negatively charged phospholipids to be exposed. This negatively charged surface is the template for full thrombin generation also involving FVIII and FIX. The absence of an increased hemostatic effect of the higher dose of 720 μg/kg suggests that the system was already saturated by the 180-μg/kg dose, rendering the increased dose ineffective.

Our prior work revealed no hemostatic effect of rFVIIa when it was used as a sole agent in warm, noncoagulopathic animals with the same grade V injury described in this study.[14] Three hypothetical explanations for a lack of effect were surmised. These included use of the drug as the sole hemostatic agent, relative hypotension of the cold, diluted animals, and suboptimal dosing of the drug in the pig model. The results of this study suggest that in coagulopathic pigs with severe venous and parenchymal liver injuries, 180 μg/kg is an adequate dose of the drug when it is used as an adjunct. Use of the drug as sole therapy in this massive liver injury and the normotensive state of the warm nondiluted animals remain likely explanations for the lack of an effect in the prior study.

Thrombin-antithrombin complexes are an indirect measure of thrombin generation. As thrombin is formed, antithrombin III complexes with it as part of a negative-feedback loop. TATs increased significantly in all three groups over the course of the study. This indicates that TATs were formed independent of rFVIIa. TATs were significantly elevated in the treatment groups relative to the control group. Despite the fact that elevation of TATs has been associated with thrombogenic situations and disseminated intravascular coagulation,[22–24] the absence of decreased platelet counts and fibrinogen levels in the treatment groups provides strong evidence that rFVIIa did not induce any systemic activation of the coagulation system or disseminated intravascular coagulation. The data suggest that rFVIIa resulted in enhanced thrombin formation only at the site of injury. The fact that TAT formation was not dose dependent is in accordance with other data from this study indicating that the system was saturated by the 180-μg/kg dose.

Despite the use of a supratherapeutic dose of rFVIIa, there was no evidence of pathologic coagulation by laboratory parameters or lung histology. These findings are consistent with prior studies in humans and animals.

The current study represents the largest trial reporting the effects of rFVIIa in pigs and the first trauma study to examine the effects of dose escalation. The study is weakened by the large variability in blood loss and resuscitation inherent in

uncontrolled hemorrhage models. This resulted in variable dilutional effects, explaining the large standard deviations seen in the coagulation parameters, especially at 1 hour and at the end of the study. Because of large standard deviations, the treatment groups were combined to show a decrease in post-treatment blood loss compared with the control group. This was warranted because there was no measurable physiologic or laboratory difference between the treatment groups except for FVII:C, which confirmed that different doses were given.

This study contributes to the increasing body of animal and human data suggesting that rFVIIa is efficacious and safe in the setting of trauma and hemorrhagic shock. Adequate preliminary data exist to support a randomized prospective study comparing rFVIIa to placebo as adjunctive therapy in human trauma patients with hemorrhagic shock, ongoing bleeding, and coagulopathy.

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DISCUSSION

Dr. Steven N. Vaslef (Durham, North Carolina): This is a follow-up study to one in which this group demonstrated that a dose of 180 μg/kg of recombinant factor VIIa significantly decreased bleeding as compared with normal saline control when used as an adjunct to abdominal packing in a liver injury model in swine. The present study was performed to confirm the efficacy of recombinant factor VIIa in reducing blood loss from grade V liver injuries in cold coagulopathic pigs when used as an adjunct to packing in grade V liver injuries. It also seeks to determine the optimal dose of recombinant factor VIIa after such an injury. The article is well written, easy to follow, and a pleasure to read.

I have several comments and questions. First, there are two possible mechanisms of action postulated to explain how recombinant factor VIIa promotes coagulation. One is a tissue factor–dependent process in which recombinant factor VIIa is combined with tissue factor to generate factors IXa and Xa to ultimately form a clot.

The second is a tissue factor–independent process in which recombinant factor VIIa is thought to bind to the activated platelets and generate thrombin in the absence of tissue factor. It is this latter tissue factor–independent process that may result in a more efficient and greater thrombin burst after the administration of high doses of recombinant factor VIIa.

My questions relate to the doses you used. The conventional dose approved by the Food and Drug Administration in hemophilia is 90 μg/kg, yet you compared 180-μg/kg dosages to 720-μg/kg dosages and found no difference.

"If some is good, more is better" seems to be the working hypothesis. Can you speculate on whether the concomitant administration of platelets may have enhanced the thrombin burst in the tissue factor–independent pathway of these doses? Why didn't you use the conventional dose of 90 μg/kg?

Second, my pharmacist tells me that a single dose of recombinant factor VIIa would cost about $8,800 for a 70-kg person using the conventional amount of 90 μg/kg. At the doses you investigated, the cost would range from $17,600 to over $70,000 for a single dose.

There are reports in the literature of multidose therapy. From a clinical and cost perspective, when would you use recombinant factor VIIa versus aggressive resuscitation with red cells, fresh frozen plasma, platelets, and cryoprecipitate?

This brings me to my next question. Wouldn't a better control group have been resuscitation with shed blood and blood products rather than crystalloid solution as current standard practice dictates in severe hemorrhage?

Finally, the authors' conclusion in the article that adequate data exist to support a randomized prospective study in humans is probably premature, given that the optimal dose is still unclear to me. A head-to-head comparison with current standard therapy has not been performed, and ultimate outcomes have not been proven to be better.

I enjoyed the article. I thank the Association for the privilege of the floor.

Dr. Ron Gross (Hartford, Connecticut): The model you used had your swine injured and then standard therapy as prescribed by the article delivered within 5 minutes. Realistically speaking, even with grade V injuries who do manage to make it to the emergency department and then the operating room, we're looking at approximately 15 to 20 minutes under the best-case scenarios and the best of all worlds. Given the limitations of swine bleeding at that rate, is there any way that you can redesign this to get closer to real-time injuries?

Dr. James G. Tyburski (Detroit, Michigan): I also enjoyed the presentation, and have a question about the model. It seems that you isovolumically depleted the pigs, and in this case didn't you actually lower their factor VII levels that were circulating?

Is this in a model that depletes factor VII and then shows that if you replace it, they do better? Maybe I missed it in the presentation, but were there any baseline activities of the factor VII of the treatment group versus what a control pig would have if it was not bled at all, in other words, the factor VII levels compared with normal?

Dr. Erik S. Barquist (Miami, Florida): I noticed some of my colleagues have gotten up to question this model, and I think there's some confusion. I'm sure that Dr. Schreiber can defend himself, but this is a very nicely performed model, and it wasn't meant to be a mimicry of what we see in clinical practice.

This is a basic science model. I had nothing to do with the design of this model, nor am I at the same institution, but I think this is a very well-designed model, which is designed to give us some insights into how factor VII works, and this is where we start with a product like this. Then, later on, we move on to perhaps more realistic models and then eventually, after time, to human trials.

I think the criticisms of this model don't reflect the fact that what we're trying to do here is understand how this protein works. I think this was a very nice article, and I congratulate the authors on their hard work.

Dr. Martin A. Schreiber (closing): I'd like to thank everybody for their comments, and I hope I can answer your questions. The first question is, Why did we use 180 μg/kg? As was correctly pointed out, one of the recommended doses was 90 μg/kg.

However, we chose to use 180 μg/kg because of potential species differences between pigs and humans. Recombinant factor VIIa is a human drug that is not 100% bioavailable in pigs; therefore, to take this effect into consideration, we doubled the dose.

The second question concerned the delivery of platelets. Recombinant factor VIIa has actually been used in patients who are thrombocytopenic.

It is one of the indications for its use, and also for patients with platelet disorders. However, we do hypothesize that in thrombocytopenic patients, giving platelets would help. This has not been done, and I can't answer this question, but I do agree that that would probably be effective.

The issue was raised about cost. Cost is a major issue with this drug; however, in the patient that I showed you, the cost of resuscitating that patient, the amount of blood products that he received, and the amount of effort that was put forth on several operations would probably equal the cost of several drug administrations.

Therefore, by decreasing the number of operations, the amount of blood transfused, the amount of effort, and the long-term potential for acute respiratory distress syndrome and multiple-organ failure by reducing blood loss, this drug could actually save money.

The next question was concerned with use of recombinant factor VIIa versus standard therapy. Currently, those of us who are trying to design a prospective randomized trial in humans are planning on using it in patients who are undergoing massive transfusion and who have ongoing blood loss.

169

LACTATED RINGER'S IS SUPERIOR TO NORMAL SALINE IN UNCONTROLLED HEMORRHAGIC SHOCK

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Lactated Ringer's (LR) and normal saline (NS) continue to be used interchangeably for the resuscitation of hemorrhagic shock in some institutions. We hypothesized that, aside from hyperchloremic acidosis, the effects of LR versus NS resuscitation would be similar in a swine model of uncontrolled hemorrhage. Twenty swine weighing a mean of 37 kg underwent invasive line placement, midline celiotomy and splenectomy. Following a 15-minute stabilization period, a baseline mean arterial pressure (MAP) was recorded followed by the creation of a grade V liver injury. The animals bled freely for 30 minutes after which blood loss was measured. The swine were then blindly randomized to receive LR versus NS at 165 cc/min to achieve and maintain the baseline MAP for 90 minutes. Laboratory values were obtained every 30 minutes throughout the 2-hour study. Initial blood loss was 22 cc/kg in the LR group and 25 cc/kg in the NS group ($p = 0.54$). Animals required 126 +/- 21 cc/kg of fluid in the LR group as compared to 256 +/- 46 cc/kg in the NS group ($p = 0.04$). The urine output was higher in the NS group (47 +/- 12 cc/kg vs. 19 +/- 4 cc/kg, $p = 0.04$). Laboratory values obtained at 2 hours are shown in the table.

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RESUSCITATION WITH LACTATED RINGER'S DOES NOT INCREASE INFLAMMATORY RESPONSE IN A SWINE MODEL OF UNCONTROLLED HEMORRHAGIC SHOCK

Purpose: Lactated Ringer's (LR) increases circulating activated neutrophils (PMNs) in large animal models of controlled hemorrhagic shock. Our hypothesis was swine resuscitated with LR would exhibit increased markers of dysfunctional inflammation compared to normal saline (NS) in a model of uncontrolled hemorrhagic shock (UHS).

Methods: Swine weighing approximately 40 kg were randomized into 4 groups. 1) Control animals were sacrificed immediately following induction of anesthesia to obtain baseline data. 2) Sham animals underwent line placement, laparotomy and 2 hours of anesthesia. 3 & 4) UHS animals underwent laparotomy, grade V liver injury and blinded resuscitation with LR or NS. Animals were sacrificed following resuscitation and maintenance of baseline blood pressure for 1.5 hours. Lung tissues were harvested immediately after sacrifice. Tissue levels of IL-6, G-CSF, and TNF-alpha mRNA were determined using QRT-PCR. Sections of lung were stained with anti-myeloperoxidase antibody and examined for the presence of PMNs within the alveolar walls. Comparisons between groups were made with independent-samples t-tests using SPSS software.

Results: Cytokine analysis showed no significant difference in IL-6 expression in any group. In LR and NS resuscitated swine, G-CSF and TNF-alpha gene expression were elevated but not different from each other. Both the LR and NS groups had significantly more alveolar PMNs present compared to controls, $p<0.01$, and shams, $p<0.05$, but were not different from one another, $p = 0.83$.

Cytokine mRNA Expression			
	IL-6	G-CSF	TNF-alpha
LR	0.06 ± 0.06	4.8 ± 4.3	8.4 ± 5.8
NS	0.06 ±0.04	5 ±10.8	4.8 ± 2.9
p-value	0.99	0.96	0.1

MPO Staining	
	PMNs/hpf
Control	6.9 ± 1.3
Sham	11.8 ± 1.6
LR	17.2 ± 6.9
NS	16.6 ± 4.9

Conclusions: Contrary to previous studies, LR resuscitation does not increase pro-inflammatory cytokines or alveolar neutrophils compared to NS resuscitation in our model of uncontrolled hemorrhagic shock.

Objective: Hypercoagulability is a major source of morbidity and mortality following injury. A resuscitation regimen that modulates this coagulopathy may prove beneficial. We sought to evaluate the effects of lactated Ringers (LR) and Hextend (HEX) on the resuscitation of uncontrolled hemorrhagic shock. **Methods:** Twenty swine underwent invasive line placement, midline celiotomy and splenectomy. Following a 15-minute stabilization period, a baseline mean arterial pressure (MAP) was determined, followed by the creation of a Grade V liver injury. After 30 minutes of uncontrolled hemorrhage, the initial blood loss was measured. The swine were then blindly randomized to receive LR or HEX to achieve and maintain the baseline MAP for 90 minutes post-injury. Laboratory values were measured at 30-minute intervals. A control group (CG) received no resuscitation. **Results:** Similar injury patterns were obtained with an initial blood loss of 22 cc/kg in both groups (p=0.97). Secondary blood loss following resuscitation was 3.7+/-1.7 cc/kg in the LR group and 4.7+/-1.1 cc/kg in the HEX group (p=0.1). Fluid requirements were 119+/-78 cc/kg in the LR group and 40+/-21 cc/kg in the HEX group (p=0.01). The urine output was 21+/-13 cc/kg in the LR group and 10+/-4 cc/kg in the HEX group (p=0.03). Laboratory values at study completion are shown in the table. Thrombelastography (TEG) R values represent the onset of clotting and values <3.7 minutes indicate hypercoagulability.

	CG	LR	HEX	*p* Values		
				LR v. CG	HEX v. CG	HEX v. LR
Hematocrit	25+/-2	15+/-3	14+/-3	<0.01	<0.01	NS
Platelets	392+/-55	322+/-92	165+/-98	NS	<0.01	<0.01
PTT	17+/-3	16+/-2	22+/-7	NS	0.05	0.02
PT	12+/-0.4	14+/-0.7	16+/-1	<0.01	<0.01	<0.01
Fibrinogen	210+/-52	161+/-42	112+/-33	NS	<0.01	<0.01
TEG R Value	1.7+/-0.8	1.7+/-0.6	2.8+/-1.1	NS	0.05	0.03

Conclusions: Resuscitation with HEX results in a decreased fluid requirement and attenuation of the hypercoagulability following severe liver injury without increasing blood loss.